Evaluation of Low-Phytate Corn and Barley on Broiler Chick Performance

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ABSTRACT Grains produced by low-phytate barley and corn isolines homozygous for each species' respective *low phytic acid 1-1* allele were compared to grain produced by near-isogenic normal or wild-type barley and corn in broiler chick feeds. Cobb × Cobb (384) chicks were used in a 10-d study. A randomized complete block design with a factorial arrangement of 2 × 2 × 3 was used with 4 replicates (8 chicks / replicate) per treatment. Twelve isocaloric and isonitrogenous treatment diets were formulated to contain 2 types of grain (barley and corn), 2 levels of grain (40% and 60%), and 3 sources of available P (wild-type grain, wild-type P-supplemented grain, and low-phytate grain). Growth parameters, bone parameters, total bone mineral, and apparent digestibilities were measured. The mean growth and bone responses were 1) higher for barley diets compared to corn diets, 2) higher for 60% grain inclusion compared to 40%, 3) higher for low-phytate compared to wild-type grains, and 4) not different for low-phytate compared to P-supplemented wild-type grain diets. Chicks fed low-phytate-based diets excreted 33 and 43% less P than chicks fed wild-type and P-supplemented wild-type diets, respectively. Correlations between percentage bone ash, total bone ash, and bone strength indicated a strong relationship and appear to support the use of bone strength analysis as a simpler method than ash content determination as an indication of P status. Feeding low-phytate grains will reduce the need for supplemental P in chick diets.

(*Key words*: chick, low-phytate barley, low-phytate corn, phosphorus, phytic acid)

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INTRODUCTION

A prerequisite to accurate ration formulation is knowledge of phosphorus availability of natural feedstuffs. In commonly used cereal grain feedstuffs, such as barley and corn, the availability of P is approximately 30 to 40% (Nelson et al., 1968; National Research Council, 1994). This low availability of P is due to the fact that, for 65 to 75% of cereal grain and oil seed meal, total P is found as phytic acid P, a compound poorly hydrolyzed by nonruminant animals (Pomeranz, 1973; O'Dell and de Boland, 1976). Phytic acid P and other minerals bound to phytic acid are poorly available because nonruminants do not have sufficient quantities of endogenous intestinal bacterial phytase, the enzyme needed to break down phytic acid (McCuaig et al., 1972; Iqbal et al., 1994). Animal waste P can ultimately contribute to water pollution and eutrophication (Miller et al., 1974).

The concentration and availability of P in the diet are the two most important factors that affect retention of P. To meet the P requirement of the animal, sources of inorganic P are often supplemented to the diet, which unfortunately increases feed cost and contributes to P pollution. Scientific research has resulted in ways to increase the availability of feedstuff P. One method is addition of phytase enzymes to facilitate the release of P from the feed (Simons et al., 1990; Ravindran et al., 1995; Kornegay, et al., 1996; Mitchell and Edwards, 1996; Gordon and Roland, 1997). Enzyme addition to practical diets increases feed cost. Moreover, enzyme supplementation requires special handling because most commercial phytases tend to denature during the pelleting process. Another approach is to reduce the amount of phytic acid and increase available P in the seeds via genetic means. Low-phytic acid genotypes of barley and corn have been developed that produce seed with reductions in phytic acid P ranging from 50 to >90% (Raboy and Gerbasi, 1996; Larson et al., 1998; Raboy et al., 2000, 2001). In grain produced by these genotypes, most of the P is stored as inorganic P instead of phytate P. Theoretically, using these mutants should reduce P excretion by animals without any additional cost or effort.

Recent research has demonstrated the effectiveness of using low-phytate corn genotypes in pig or poultry feeds (Ertl et al., 1998; Douglas et al., 2000; Li et al., 2000; Spencer et al., 2000a,b; Veum et al., 2001) the potential value of using such genotypes in combination with phytase supplementation (Huff et al., 1998; Waldroup et al., 2000; Yan et

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TABLE 1. Composition of test grains (DM basis)

Ingredient	Barley WT[1]	Barley LowPhy[1]	Corn WT	Corn LowPhy
	(% of dry matter)			
DM	91.52	91.70	90.83	91.23
Ash	2.47	2.28	1.32	1.29
Crude Protein	14.10	14.21	9.79	12.46
Ether Extract	2.51	2.72	4.09	4.01
β-Glucans	3.63	3.74	0.01	0.00
Starch	55.28	55.74	62.91	64.80
Total P	0.41	0.33	0.32	0.32
Phytic Acid	0.23	0.11	0.22	0.09
Available P	0.18	0.22	0.10	0.23
Ca	0.06	0.06	0.002	0.003
Mg	0.13	0.13	.13	0.12
Zn, ppm	30.00	37.02	14.10	14.10
Cu, ppm	8.76	11.02	5.86	6.39
Mn, ppm	16.91	19.63	7.22	7.13
Arg	0.72	0.76	0.44	0.46
Cys	0.33	0.33	0.22	0.22
Lys	0.50	0.52	0.26	0.28
Met	0.25	0.24	0.18	0.19
TME (Mcal/kg)	3.09	3.12	3.60	3.53

[1]WT = wild-type, LowPhy = low-phytate.

al., 2000). Studies have also demonstrated the effectiveness of using low-phytate barley genotypes in poultry feeds (Li et al., 2001a,b). Only one study to date, utilizing trout as a model, has compared the nutritional value of normal phytate and low-phytate genotypes of corn and barley genotypes side-by-side (Sugiura et al., 1999). The objective of this study was to determine and compare the effects of low-phytate, P supplemented wild-type (nonmutant normal phytate), and unsupplemented wild-type grains of barley and corn on growth, bone characteristics, and digestibility for broiler chicks and to evaluate and determine any correlation among these response criteria.

MATERIALS AND METHODS

Test Grain Analysis

Four test grains were evaluated: grain produced by a wild-type, normal-phytate barley cultivar (*Hordeum vulgare*) (cv. Harrington); grain produced by a near-isogenic line of the same barley cultivar, homozygous for the barley *low phytic acid 1-1* allele (Larsen et al., 1998); grain produced by a wild-type, normal phytate dent corn (*Zea mays*) hybrid, and grain produced by near-isohybrid homozygous for the corn *low phytic acid 1-1* allele (Raboy et al., 2000). A subsample (approximately 100 g) of each grain was finely ground using a Wiley mill[2] (1 mm) for chemical analysis. The analytical composition of the grains is presented in Table 1. Analyses of the ground samples were performed in duplicate for dry matter, ash, crude protein, and crude fat (AOAC, 1990). Calcium, magnesium, zinc, copper, and manganese were analyzed by atomic absorption spectrophotometry[3] using standard procedures (AOAC, 1990). Total P was analyzed with a Technicon Autoanalyzer[4] using method N-4C (Kraml, 1966). Phytic acid P was measured using a modified phytic acid method, which precipitates the ferric salt from the inorganic P and quantifies the ferric phytate with a Technicon Autoanalyzer (Xu et al., 1992). Nonphytate P was calculated as the difference between total P and phytic acid P and was considered, for the purposes of this experiment, to be available (available P). Amino acid content was measured by sample hydrolysis and quantification using HPLC (AOAC, 1990). β-Glucan content was determined with the Megazyme mixed linkage β-glucan assay kit, which uses purified lichenase and β-glucosidase enzymes and colorimetric spectroscopy[5] (Megazyme International Ireland Ltd., 1998a). Total starch content was determined with the Megazyme total starch assay kit, which uses α-amylase and amyloglucosidase enzymes and colorimetric spectroscopy (Megazyme International Ireland Ltd., 1998b). True metabolizable energy was determined to be similar within test grain types when using a standard method (Sibbald, 1989).

Experimental Diets

The 4 grains were ground using a Hobart grinder[6] (1 mm) and mixed into formulated diets, and levels of casein, cellulose, and starch were adjusted to achieve isocaloric and isonitrogenous levels. All nongrain ingredients used in the diets were free of phytic acid to eliminate any extraneous source of phytic acid. All formulated diets contained adequate levels of all nutrients, except P, to satisfy the NRC (1994) requirements. Calcium content of the diets was kept constant (1.0%) by varying the levels of calcium carbonate and dibasic calcium phosphate.

[2]Thomas Scientific, Swedesboro, NJ.
[3]Model 3030B, Perkin-Elmer, Norwalk, CT.
[4]Technicon Autoanalyzer, Terrytown, NY.
[5]Milton Roy Co., Rochester, NY.
[6]Hobart Corp., Troy, OH.

TABLE 2. Ingredient and composition of barley treatment diets incorporating wild-type, P-supplemented wild-type, or low-phytate at 2 levels of barley

Ingredient	Barley 40% WT[1]	Barley 60% WT	Barley 40% PSWT	Barley 60% PSWT	Barley 40% LowPhy	Barley 60% LowPhy
(% as-fed)						
Wild barley	40.00	60.00	40.00	60.00	0.00	0.00
Low-phytate barley	0.00	0.00	0.00	0.00	40.00	60.00
Corn starch	28.12	16.02	28.12	16.02	27.86	15.64
Casein[2]	17.63	15.85	17.63	15.85	17.63	15.85
Cellulose[3]	7.51	1.50	7.45	1.40	7.75	1.85
Calcium carbonate	2.26	2.24	2.16	2.11	2.28	2.26
Corn oil	2.00	2.00	2.00	2.00	2.00	2.00
Potassium chloride	0.79	0.79	0.79	0.79	0.79	0.79
Arg-HCl	0.66	0.61	0.66	0.61	0.66	0.61
Vitamin-mineral premix[4]	0.25	0.25	0.25	0.25	0.25	0.25
Choline chloride	0.20	0.20	0.20	0.20	0.20	0.20
DL-Met	0.34	0.31	0.34	0.31	0.34	0.31
Sodium chloride	0.14	0.14	0.14	0.14	0.14	0.14
β-Glucanase[5]	0.10	0.10	0.10	0.10	0.10	0.10
Calcium diphosphate	0.00	0.00	0.16	0.22	0.00	0.00
Analyzed composition (DM basis)						
Crude protein (%)	24.69	25.31	24.30	24.75	23.88	24.94
Total phosphorus (%)	0.35	0.40	0.39	0.45	0.32	0.36
Phytate phosphorus (%)	0.09	0.13	0.10	0.14	0.05	0.07
Available phosphorus (%)	0.26	0.27	0.29	0.31	0.27	0.29
Calcium (%)	1.28	1.09	1.05	1.10	1.07	1.08
Magnesium (%)	0.06	0.09	0.07	0.09	0.07	0.09
Zinc (mg/kg)	167.54	116.28	117.57	144.65	122.29	132.74
Copper (mg/kg)	22.60	24.92	28.63	32.88	34.44	40.60
Manganese (mg/kg)	138.37	147.99	146.62	148.87	139.31	144.16

[1]WT = wild-type, PSWT = P-supplemented wild-type, LowPhy = low-phytate.

[2]Casein (United States Biochemical, USB, Cleveland, OH).

[3]Celfil (USB).

[4]Broiler starter premix (Nutritech, Edmonton, AB, Canada) supplied per kilogram of diet: vitamin A, 10,000 IU; vitamin D, 4,000 IU; vitamin E, 45 IU; vitamin K, 5 mg; vitamin B_1, 3 mg; vitamin B_2, 7 mg; vitamin B_6, 5 mg; vitamin B_{12}, 0.04 mg; folic acid, 1.5 mg; D-calpan, 15 mg; biotin, 0.250 mg; niacin, 50 mg; Se 0.3 mg; Co, 0.6 mg; I, 1 mg; Mo, 0.9 mg; Cu, 15 mg; Fe, 25 mg; Mn, 110 mg; Zn, 80 mg; virginiamycin, 0.010 mg.

[5]Avizyme (Enzyme Development Corporation, New York).

The treatment diets (Tables 2 and 3) were formulated to test 3 main effects: grain type (barley and corn), amounts of grain (40 and 60%), and source of P (P from wild-type grain, P from P-supplemented wild-type grain, and P from low-phytate grain). The P-supplemented diets were prepared by mixing calcium carbonate and reagent-grade dicalcium phosphate with the wild-type grain diet ingredients to achieve a level of Ca equivalent to the corresponding wild-type grain diet and a level of available P equivalent to the corresponding low-phytate grain diet. This procedure was done to determine if the results obtained using low-phytate grains were comparable to those that might be expected on a more conventional type of diet containing additional available P. The 12 treatment diets were 1) 40% wild-type barley, 2) 60% wild-type barley, 3) 40% wild-type barley supplemented with 0.03% available P, 4) 60% wild-type barley supplemented with 0.04% available P, 5) 40% low-phytate barley, 6) 60% low-phytate barley, 7) 40% wild-type corn, 8) 60% wild-type corn, 9) 40% wild-type corn supplemented with 0.02% available P, 10) 60% wild-type corn supplemented with 0.03% available P, 11) 40% low-phytate corn, and 12) 60% low-phytate corn. Diet samples were analyzed for dry matter, ash, total P, phytic acid P, available P, Ca, Mg, Zn, Cu, and Mn by using the test grain analysis procedures described previously.

Feeding Regimen

All chicks used in this study were hatched from eggs obtained from Foster Farms.[7] Four hundred sixty-four commercial broilers (Cobb × Cobb) at 1 d of age were available for the trial. A total of 384 mixed sex birds were selected based on uniform BW, and 32 birds were assigned to each treatment diet with 8 birds per cage. The weight of each cage of birds was adjusted by chick selection to equalize average cage weight and variance. Each cage of birds was considered an experimental unit. The trial was conducted in an environmentally controlled room maintained at 21°. Birds were housed in Petersime battery-brooders[8] and provided with 24 h of constant light each day. Birds were fed a commercial starter diet for 3 d. Birds at age 4 d of age were started on 1 of the 12 treatment diets. Diets in mash form and water were provided ad libitum for 10 d.

Sample Collection

Feed and bird weights were recorded at d 0, 6, and 10 of the feeding period. Mortality of 1 bird per day was

[7]Turlock, CA.

[8]Petersime, Gettysburg, OH.

TABLE 3. Ingredient and composition of corn treatment diets incorporating wild-type, P-supplemented wild-type, or low-phytate at 2 levels of corn

Ingredient	Corn 40% WT[1]	Corn 60% WT	Corn 40% PSWT[1]	Corn 60% PSWT	Corn 40% LowPhy[1]	Corn 60% LowPhy
(% as-fed)						
Wild-type corn	40.00	60.00	40.00	60.00	0.00	0.00
Low-phytate corn	0.00	0.00	0.00	0.00	40.00	60.00
Corn starch	22.29	7.48	22.29	7.48	22.76	8.20
Casein[2]	19.29	18.11	19.29	18.11	19.29	18.11
Cellulose[3]	11.74	7.83	11.70	7.76	11.26	7.11
Calcium carbonate	2.31	2.32	2.26	2.22	2.32	2.33
Corn oil	2.00	2.00	2.00	2.00	2.00	2.00
Potassium chloride	0.79	0.79	0.79	0.79	0.79	0.79
Arg-HCl	0.66	0.62	0.66	0.62	0.66	0.62
Vitamin-mineral premix[4]	0.25	0.25	0.25	0.25	0.25	0.25
Choline chloride	0.20	0.20	0.20	0.20	0.20	0.20
DL-Met	0.24	0.16	0.24	0.16	0.24	0.16
Sodium chloride	0.14	0.14	0.14	0.14	0.14	0.14
β-Glucanase[5]	0.10	0.10	0.10	0.10	0.10	0.10
Calcium diphosphate	0.00	0.00	0.10	0.18	0.00	0.00
Analyzed composition (DM basis)						
Crude protein (%)	22.75	23.94	23.31	23.40	23.50	24.00
Total phosphorus (%)	0.32	0.36	0.33	0.39	0.32	0.37
Phytate phosphorus (%)	0.09	0.12	0.09	0.13	0.04	0.06
Available phosphorus (%)	0.23	0.24	0.24	0.26	0.28	0.31
Calcium (%)	1.07	0.99	1.10	1.03	0.98	1.00
Magnesium (%)	0.07	0.08	0.06	0.08	0.06	0.09
Zinc (mg/kg)	123.36	102.42	138.55	109.82	120.26	128.83
Copper (mg/kg)	38.68	14.68	18.85	20.49	17.86	16.92
Manganese (mg/kg)	142.83	123.71	132.70	145.69	139.35	143.46

[1]WT = wild-type, PSWT = P-supplemented wild-type, LowPhy = low-phytate.

[2]Casein (United States Biochemical, USB, Cleveland, OH).

[3]Celfil (USB).

[4]Broiler starter premix (Nutritech, Edmonton, AB, Canada) supplied per kilogram of diet: vitamin A, 10,000 IU; vitamin D, 4,000 IU;vitamin E, 45 IU; vitamin K, 5 mg; vitamin B_1, 3 mg; vitamin B_2, 7 mg; vitamin B_6, 5 mg; vitamin B_{12}, 0.04 mg; folic acid, 1.5 mg; D-calpan, 15 mg; biotin, 0.250 mg; niacin, 50 mg; Se 0.3 mg; Co, 0.6 mg; I, 1 mg; Mo, 0.9 mg; Cu, 15 mg; Fe, 25 mg; Mn, 110 mg; Zn, 80 mg; virginiamycin, 0.010 mg.

[5]Avizyme (Enzyme Development Corporation, New York).

observed on d 2, 6, and 8, and feed and bird weights were recorded. Feces were collected quantitatively in pans beneath the cages from d 6 to d 10 and manually scraped into plastic bins on d 10. The scrapings, however, contained both feces and spilled feed. To remove the majority of spilled feed, the feces were separated by particle size through a sieve (3 mm). The large-sized feces fraction (approximately 82%, wt/wt) was dried for several days in an oven at 65°C to a constant weight and ground using a Wiley mill (1 mm). The fine-sized uniform fraction (predominantly spilled feed) was weighed, and the result was used in feed intake calculation. Visual inspection of the fine-sized fraction provided qualitative evidence that this fraction was mainly spilled feed. However, some fecal particles might have been in this fraction. Feces samples were analyzed for dry matter, ash, total P, phytic acid P, available P, Ca, Mg, Zn, and Cu using the grain analysis procedures previously described.

The feed-to-gain ratio was calculated using the feed intake from d 0 through 10 (0 to 10 d) divided by weight gain (0 to 10 d) on a per cage basis. Feces excreted were calculated using the large-sized fraction weight for the period from d 6 through 10 (6 to 10 d). Final bird weights were recorded at d 10 prior to random selection of 6 birds per cage for euthanasia via cervical dislocation. Both tibiae from each bird were dissected and cleaned of all adhering tissues. Bones were then frozen at −12°C to be later analyzed. After thawing to room temperature, breaking force was determined for 1 randomly selected tibia per bird using a texture analyzer.[9] Briefly, the texture analyzer measures force in compression using a 3-point bending rig and a 50-kg loading cell. Before securing the rig, the base plate was adjusted so the 2 securing blades were 5 cm apart. The base plate was then placed onto the machine's platform and secured in a position that enabled the upper blade to be equidistant from the 2 lower blades. The upper probe was then raised above the rig, and the sample was placed centrally over the lower supports. Once the trigger force was initiated, the force was increased until the bone fractured and fell into 2 pieces. This measurement was recorded as the peak force (bone strength) required to fracture the sample (Anon, 1996). After bone strength was determined, all bones, including those broken during strength testing, were placed in individual ceramic pans and dried in an oven at 100°C overnight to determine dry matter content. The dried tibiae from each cage were ashed in the same ceramic pans at 575°C overnight to determine

[9]Texture Technologies Corporation, Scarsdale, NY.

TABLE 4. Least squares means of growth and bone responses and *P*-values using ANOVA model for low-phytate and wild-type diets

Item	Final weight 10 d (g/bird)	Weight gain 0–10 d (g/bird)	Feed intake 0–10 d (g/bird)	Feed intake 6–10 d (gDM/bird)	Feed to gain ratio 0–10 d (g:g)	Feces 6–10 d (gDM/bird)	Tibiae bone ash 10 d (% DM)	Total tibiae bone ash 10 d (g/bird)	Tibia bone strength 10 d (kg)
Least squares means									
Grain									
Barley	205.4	131.3	203.7	87.6	1.57	20.2	37.1	0.264	4.48
Corn	194.2	120.6	192.9	84.2	1.61	17.9	35.2	0.243	4.02
Amount									
40%	185.8	112.3	185.4	79.8	1.67	17.8	35.4	0.233	3.69
60%	213.8	139.6	211.2	92.0	1.52	20.3	36.9	0.274	4.80
P source									
Low-phytate	212.8	138.1	212.9	93.0	1.55	20.9	38.4	0.289	5.13
Wild-type	186.8	113.8	183.7	78.8	1.64	17.2	33.9	0.219	3.37
P-value									
Grain	0.002	0.002	0.002	0.067	0.066	<0.001	0.001	0.006	0.014
Amount	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
P source	<0.001	<0.001	<0.001	<0.001	0.001	<0.001	<0.001	<0.001	<0.001
Pooled SE	2.35	2.24	2.25	1.27	0.017	0.33	0.24	0.005	0.121

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ash content. The ashes were dissolved in concentrated HCl and transferred to a volumetric flask for P, Ca, Mg, Zn, Cu, and Mn determination using the grain analysis procedures described previously. Both tibiae were removed from a subset of 5 to 6 birds per cage and ashed collectively. The results are reported on a per bird basis.

Digestibility was determined for each mineral using the chemical analysis results of feed and feces. Because endogenous fecal values were not determined, the values given represent apparent digestibility rather than true digestibility.

Statistical Analysis

In order to compare the effects of P-supplemented wild-type diets with the low-phytate diets, levels of available P for the wild-type diets were formulated to match those of the low-phytate diets. The original model investigated all 12 treatment diets. The original model for the ANOVA was

$$Y_{ijkl} = \mu + \alpha_i + \beta_j + \gamma_k + (\alpha\beta)_{ij} + (\beta\gamma)_{jk} + (\alpha\gamma)_{ik} + \varepsilon_{ijkl}$$

where the main effects were grain type (α), amount of grain (β), and P source (γ); and i = 1 if barley, 2 if corn; j = 1 if 40% grain in diet, 2 if 60% grain in diet; and k = 1 if wild-type, 2 if P-supplemented wild-type, 3 if low-phytate.

Discrepancies were found in the chemically analyzed values of the available P in the diets compared to the preplanned formulated levels, and so direct comparisons between P-supplemented and low-phytate diets were not performed. Chemically analyzed values were used in all statistical analyses. The P-supplemented wild-type barley group was slightly overformulated for available P, and the P-supplemented wild-type corn group was slightly underformulated for available P. Analyzed compositions are presented in Tables 2 and 3. The model was revised and separated into 2 models. One model included wild-type and low-phytate diets, and another model, with dietary available P concentration of the treatment diet as the covariate, included P-supplemented wild-type and low-phytate diets.

To test the differences in responses between wild-type and low-phytate diets, the model used for the ANOVA was the same as stated above.

$$Y_{ijkl} = \mu + \alpha_i + \beta_j + \gamma_k + (\alpha\beta)_{ij} + (\beta\gamma)_{jk} + (\alpha\gamma)_{ik} + \varepsilon_{ijkl}$$

where the main effects were grain type (α), amount of grain (β), and P source (γ); and i = 1 if barley, 2 if corn; j = 1 if 40% grain in diet, 2 if 60% grain in diet; and k = 1 if wild-type, 2 if low-phytate.

To test the differences in responses between P-supplemented and low-phytate diets, actual dietary available P concentration was used as a covariate variable to account for the differences in available P in the diets. The model for the ANCOVA was

$$Y_{ijkl} = \mu + \alpha_i + \beta_j + \gamma_k + (\alpha\beta)_{ij} + (\beta\gamma)_{jk} + (\alpha\gamma)_{ik} + \tau X_{ijk} + \varepsilon_{ijkl}$$

where the main effects were grain type (α), amount of grain (β), and P source (γ); and τX = available P concentration is the covariate for grain, amount, and P source; and i = 1 if barley, 2 if corn; j = 1 if 40% grain in diet, 2 if 60% grain in diet; and k = 1 if P-supplemented wild-type, 2 if low-phytate.

Statistical analyses of the treatment diets were by 3-factor ANOVA and 3-factor ANCOVA. The 3 main factors were type of grain, amount of grain in diet, and P source. Statistical significance was considered at $P < 0.05$ (Snedecor and Cochran, 1980). Body weight, weight gain (0 to 10 d), feed intake (0 to 10 d), feed-to-gain ratio (0 to 10 d), feed intake (6 to 10 d), amount of feces (6 to 10 d), percentage bone ash, total bone ash, bone strength, bone mineral, and apparent digestibility results were analyzed as a $2 \times 2 \times 2$ factorial arrangement of treatments with the diet sources using PROC GLM, the general linear models procedure (SAS, 1985).

TABLE 5. Least squares means of growth and bone responses and *P*-values using ANCOVA model for low-phytate and P-supplemented wild-type diets

Item	Final weight 10 d (g/bird)	Weight gain 0–10 d (g/bird)	Feed intake 0–10 d (g/bird)	Feed intake 6–10 d (gDM/bird)	Feed to gain ratio 0–10 d (g:g)	Feces 6–10 d (gDM/bird)	Tibiae bone ash 10 d (% DM)	Total tibiae bone ash 10 d (g/bird)	Tibia bone strength 10 d (kg)
Least squares means									
Grain									
Barley	221.3	144.8	217.4	97.4	1.48	22.3	38.3	0.286	4.69
Corn	210.2	129.1	204.3	87.9	1.62	18.9	37.6	0.281	5.16
Amount									
40%	193.7	122.4	198.0	81.8	1.67	18.8	37.8	0.280	5.30
60%	228.9	151.5	223.6	103.6	1.43	22.4	38.0	0.287	4.55
P Source									
Low-phytate	215.0	138.6	212.9	95.1	1.52	21.2	38.0	0.280	4.69
PSWT[1]	207.6	135.3	208.8	90.2	1.58	20.0	37.8	0.287	5.16
P-value									
Grain	0.121	0.164	0.292	0.241	0.074	0.102	0.538	0.839	0.487
Amount	0.082	0.099	0.187	0.089	0.059	0.243	0.883	0.856	0.490
P Source	0.437	0.681	0.652	0.415	0.308	0.448	0.832	0.747	0.364
Pooled SE	7.06	6.18	6.87	4.49	0.043	0.83	0.65	0.014	0.388

[1]PSWT = P-supplemented wild-type.

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When the model was found to be statistically significant, interactions between main factor effects were investigated. When an interaction effect was statistically significant, the data were graphed in the form of a treatment means plot and were determined to be important if the lines intersected or the signs of the 2 slopes were different. If no interaction was present or the interaction did not meet the criteria of importance, each main factor effect using the ANOVA and ANCOVA was studied. Interest in the ANCOVA model primarily focused on the P source factor effect. If the interaction was important, individual contrasts using a cell means model were performed to further investigate the factor effects.

Correlation coefficients were calculated to determine the relationships among growth responses, bone responses, and available P intake. Calculations were performed using several sets of data including the entire data and partitioned data based on 6 categories of grain and P source. The categories of six grain and P source were wild-type, P-supplemented wild-type, and low-phytate as the P sources and with barley and corn as the grains. Grains are incorporated at 40 and 60% of the diet for each category of grain and P source. Statistical analysis was performed using the PROC CORR correlation procedure (SAS, 1985).

RESULTS

Growth and Bone Responses

The results from the ANOVA of growth and bone responses for low-phytate and wild-type diets are presented in Table 4. Significant differences in all responses were found for all main effects, except for the feed-to-gain ratio response for the grain effect. Differences in the least squares means indicated performance was improved significantly by feeding diets prepared with barley over corn, diets with 60% grain over 40%, and diets prepared with low-phytate grain over wild-type grain. Feed intake (6 to 10 d) had a grain x P source interaction ($P = 0.028$). Further analyses using a cell means model showed that feed intake between low-phytate barley and low-phytate corn was not significant (102.7 vs. 103.2), but feed intake was different ($P = 0.002$) between wild-type barley and wild-type corn (91.9 vs. 82.5). This difference between low-phytate and wild-type corn and barley explains this interaction.

The results from the ANCOVA of growth and bone responses for low-phytate and P-supplemented wild-type diets are presented in Table 5. Available P was used as a covariate in this statistical model. No differences in growth and bone characteristics were observed for any main effect.

Correlation analysis using data from all 12 treatment diets showed ($P < 0.001$) strong relationships ($r \geq 0.77$) between all pairwise comparisons of available P intake, final BW, weight gain, feed intake, percentage bone ash, total bone ash, bone strength, total bone P, and total bone Ca. Available P intake correlations with growth and bone responses were always higher than correlations of dietary available P concentration with growth and bone responses. Dietary phytic acid P levels were poorly correlated with growth and bone responses. A higher correlation was observed between total bone ash and bone strength ($r = 0.95$) compared with percentage bone ash and bone strength ($r = 0.90$). To test the robustness of the correlations, pairwise comparisons were partitioned into categories of 6 grain and P source. The 6 categories were wild-type, P-supplemented wild-type, and low-phytate as the P sources and barley and corn as the grains. The pairwise correlation results of the 6 categories were generally similar to the results from the pooled data. However, of particular interest for all categories, high correlations were found for BW compared with available P intake ($r > 0.90$) and bone strength compared to total bone ash ($r > 0.85$ except for corn P-supplemented wild-type where $r = 0.74$).

Bone Minerals

Results from the ANOVA of total tibiae bone mineral for low-phytate and wild-type treatment diets are presented in

TABLE 6. Least squares means of tibia bone minerals (mg/bird) and *P*-values using ANOVA model for low-phytate and wild-type diets

	Total P	Ca	Mg	Zn	Cu
Least squares means					
Grain					
Barley	53.0	95.1	1.28	0.52	0.008
Corn	48.5	86.2	1.20	0.46	0.007
Amount					
40%	46.4	82.6	1.09	0.47	0.007
60%	55.2	98.8	1.39	0.52	0.008
P Source					
Low-phytate	58.5	104.6	1.46	0.58	0.008
Wild-type	43.1	76.7	1.01	0.40	0.007
P-value					
Grain	0.002	0.004	0.090	<0.001	0.663
Amount	<0.001	<0.001	<0.001	0.001	0.012
P Source	<0.001	<0.001	<0.001	<0.001	<0.001
Pooled SE	0.93	1.98	0.034	0.010	0.0002

Table 6. Significant differences were found for all main effects (grain, amount, and P source), except for the Mg and Cu grain effect. Mineral content increased by feeding diets prepared with barley over corn, diets with 60% grain over 40%, and diets prepared with low-phytate grain over wild-type grain.

The results from the ANCOVA, where dietary available P was used as a covariate, for P-supplemented wild-type and low-phytate treatment diets are presented in Table 7. No differences in bone minerals were found for any main effect.

Apparent Digestibility of DM and Minerals

Results from the ANOVA for low-phytate and wild-type treatment diets are given in Table 8. The interactions were amount × P source for Ca digestibility ($P = 0.003$), for Zn digestibility ($P = 0.002$), and for Mn digestibility ($P = 0.031$), and grain type × P source for total Ca in feces ($P < 0.01$) and for Cu digestibility ($P < 0.001$). All other main factor effects were tested. Significant differences for main effects were found in which feeding diets prepared with corn had higher apparent digestibility of DM and lower apparent digestibility for phytic acid P compared to barley-based diets. Feeding diets prepared with 60% grain resulted in decreased digestibility of ash, total P, and phytic acid P compared to 40% grain diets. Low-phytate grain diets showed an increase in total P digestibility compared to wild-type diets. Birds consumed 13% more total P when fed the low-phytate diets compared to the birds fed the wild-type diets but excreted 33% less total P.

A cell means model was used to study the interactions. The interaction for grain type × P source for total Ca in the feces was explained when the difference between low-phytate barley and wild-type barley (429 mg / bird vs. 435 mg / bird) was not significant but was significant when comparing the difference between low-phytate corn and wild-type corn (461 mg / bird vs. 386 mg / bird; $P = 0.001$). A similar test for the grain type × P source for Cu digestibility showed a difference between low-phytate barley and wild-type barley (63% vs. 46%; $P = 0.005$) and a difference between low-phytate corn and wild-type corn (15% vs. 51%; $P = 0.001$), but the differences went in the opposite directions. These interactions are interesting but not as consistent as the amount × P source interactions. The three amount × P source interactions for the Ca, Zn, and Mn digestibilities

TABLE 7. Least squares means of tibia bone minerals (mg/bird) and *P*-values using ANCOVA model for low-phytate and P-supplemented wild-type diets

	Total P	Ca	Mg	Zn	Cu
Least squares means					
Grain					
Barley	57.4	106.1	1.40	0.56	0.009
Corn	57.1	101.0	1.39	0.54	0.008
Amount					
40%	56.6	99.4	1.33	0.57	0.007
60%	57.9	107.7	1.46	0.53	0.009
P Source					
Low-phytate	56.4	102.3	1.41	0.56	0.009
PSWT[1]	58.1	104.8	1.38	0.54	0.008
P-value					
Grain	0.949	0.661	0.975	0.749	0.464
Amount	0.880	0.641	0.685	0.647	0.396
P Source	0.680	0.771	0.855	0.671	0.197
Pooled SE	3.14	6.40	0.109	0.034	0.0005

[1]PSWT = P-supplemented wild-type.

TABLE 8. Least squares means of total Ca and P consumed and in feces, apparent digestibility (%) of DM and minerals, and *P*-values using ANOVA model for low-phytate and wild-type diets

	DM (%)	Ash (%)	Total P (%)	Phytate P (%)	Total P consumed (mg/bird)	Total P in feces (mg/bird)	Ca (%)	Total Ca consumed (mg/bird)	Total Ca in feces (mg/bird)	Mg (%)	Zn (%)	Cu (%)	Mn (%)
Least squares means													
Grain													
Barley	76.9	48.5	76.4	39.9	314	74.5	56.0	983	432	34.5	29.4	54.5	28.3
Corn	78.8	48.9	75.0	25.6	289	70.9	50.2	848	423	37.9	31.1	33.0	30.0
Amount													
40%	77.8	51.3	78.5	34.4	260	54.4	55.2	871	390	39.5	35.0	51.7	32.6
60%	77.9	46.1	72.9	31.0	343	91.4	51.0	959	466	32.9	25.5	35.6	25.7
P Source													
Low-phytate	77.5	49.1	81.9	32.7	320	58.6	53.5	960	445	35.9	32.4	39.1	28.3
Wild-type	78.2	48.3	69.5	32.8	282	87.2	52.7	871	410	36.6	28.1	48.2	30.0
P-Values													
Grain	<0.001	0.728	0.074	<0.001	<0.001	0.157	<0.001	<0.001	0.529	0.003	0.650	<0.001	0.243
Amount	0.708	<0.001	<0.001	0.031	<0.001	<0.001	0.001	<0.001	<0.001	<0.001	0.015	<0.001	<0.001
P Source	0.040	0.462	<0.001	0.961	<0.001	<0.001	0.528	<0.001	0.022	0.522	0.252	<0.033	0.248
Pooled SE	0.24	0.79	0.54	1.05	4.3	1.95	0.82	14.3	10.0	0.72	2.58	2.83	1.02

TABLE 9. Least squares means of total Ca and P consumed and in feces, apparent digestibility (%) of DM and minerals, and *P*-values using ANCOVA model for low-phytate and P-supplemented wild-type diets

	DM (%)	Ash (%)	Total P (%)	Phytate P (%)	Total P consumed (mg/bird)	Total P in feces (mg/bird)	Ca (%)	Total Ca consumed (mg/bird)	Total Ca in feces (mg/bird)	Mg (%)	Zn (%)	Cu (%)	Mn (%)
Least squares means													
Grain													
Barley	77.1	47.5	76.1	39.3	356	86.3	51.9	988	469	35.6	11.6	63.5	29.1
Corn	78.4	47.4	76.7	24.5	326	78.5	52.6	959	460	36.6	47.8	18.7	27.0
Amount													
40%	77.0	46.6	80.7	31.0	301	60.7	56.3	950	422	36.8	55.0	30.6	25.7
60%	78.5	48.3	72.0	32.8	381	104.1	48.2	998	507	35.4	4.3	51.6	30.4
P Source													
Low-phytate	77.8	50.4	81.0	33.7	317	59.7	52.0	940	447	36.8	21.1	44.6	30.0
PSWT[1]	77.8	44.6	71.8	30.1	365	105.1	52.5	1008	482	35.4	38.2	37.6	26.1
P-Values													
Grain	0.658	0.985	0.872	0.022	0.346	0.729	0.903	0.736	0.889	0.829	0.041	0.006	0.743
Amount	0.440	0.848	0.198	0.851	0.108	0.217	0.338	0.714	0.404	0.848	0.063	0.372	0.648
P Source	0.560	0.185	0.008	0.442	0.049	0.011	0.911	0.288	0.475	0.694	0.185	0.539	0.428
Pooled SE	0.90	3.24	2.39	3.45	17.5	12.49	3.02	47.5	36.4	2.56	9.49	8.42	3.71

[1]PSWT = P-supplemented wild-type.

all showed a similar nonsignificant response (54 vs. 53%, 31 vs. 34%, and 30 vs. 27%, respectively) when the low-phytate diets were increased from 40 to 60% grain. However, the Ca, Zn, and Mn digestibilities all showed a decrease (57 vs. 47%, 39 vs. 17%, and 35 vs. 25%, respectively; $P < 0.0001$) when the wild-type diets were increased from 40 to 60% grain. The total phytic acid consumed per bird increased from 42 to 64 mg/bird when the low-phytate diets were increased from 40 to 60% grain and increased from 65 to 109 mg/bird when the wild-type diets were increased from 40 to 60% grain, possibly explaining the significant decrease in digestibility with the wild-type diets compared to the low-phytate diets.

The results from the ANCOVA for low-phytate and supplemented treatment diets are presented in Table 9. All main factor effects were tested. Feeding barley diets over corn resulted in increased phytic P and Cu digestibility and decreased Zn digestibility, and feeding P-supplemented diets over low-phytate resulted in increased total P digestibility. Most of the differences in the main effects in Table 8 were not in Table 9 when the wild-type diets were supplemented with P. Birds consumed 13% less total P when fed the low-phytate diets compared with birds fed the P-supplemented wild-type diets but excreted 43% less total P.

DISCUSSION

Growth and Bone Performance and P Bioavailability

The results indicated significant differences in growth and bone performance between 40 and 60% grain inclusion levels. The diets were prepared as a semi-chemically defined formulation using casein, cellulose, and starch among the main nongrain ingredients. In practical poultry diets, normal inclusion rates for barley meal and corn meal are 0 to 60% and 10 to 70%, respectively, with a large part of the balance consisting of soybean meal. Chemically defined diets may have lower palatability than practical diets, and the higher grain diets, 40 and 60% inclusion rates used in this study, helped offset the potentially poor palatability of pure chemical diets.

Growth and bone performances were improved with low-phytate grain compared to P-supplemented or wild-type grain diets. The improvement in growth and bone performance in birds fed low-phytate diets and P-supplemented diets may be attributed to fewer minerals in a phytate mineral complex (Simons et al., 1990), increased starch digestibility (Knuckles and Betschsrt, 1987), or increased availability of proteins. Protein is less available if it forms a complex with phytate. Phytate-protein complexes are less subject to proteolytic digestion than the same protein alone (Kratzer and Vohra, 1986). Responses from birds fed the low-phytate diets compared to the P-supplemented wild-type diets were mostly nonsignificant because the level of available P was similar. However, the birds fed the low-phytate diets had higher P digestibility and lower total P excretion. These results confirm the findings of other studies that lower-phytate diets dramatically decrease the release of P excretion into the environment while not affecting growth (Sugiura et al., 1999; Waldroup et al., 2000; Veum et al., 2001).

The low-phytate corn diets have been reported to increase the percentage bone ash compared to wild-type corn diets (Cromwell et al., 2000; Douglas et al., 2000; Waldroup et al., 2000). Tibia percentage bone ash is considered to be a good indicator of increased bone mineralization associated with consequent increased P and Ca availability. Percentage bone ash was found to be highly correlated with available P intake in this study ($r = 0.90$), and because percentage bone ash, total bone ash, and bone strength were found to be highly correlated, any of these responses would also be considered a good indicator. As the availability of P increased, the availability of Ca also increased, and both were deposited in the bones (Simons et al., 1990). Phosphorus and Ca together account for >50% of the bone ash content (Qian et al., 1996), and any treatment that results in their increased bioavailability will have positive effects on bone DM and ash contents. In this study, the concentrations of P and Ca in bone ash were significantly improved with the low-phytate diets, indicating P and Ca availability is higher in the low-phytate diets. Concentration of bone minerals was not different for birds fed the P-supplemented diet when compared with the low-phytate diets. This finding indicates that improvement in Ca utilization is due to the increase in available P in low-phytate diets rather than the decrease in phytic acid P.

After partitioning the data, the correlations were found to be strong across all grain type-P source categories, supporting the original correlation findings. The correlation of growth responses agrees with previous findings (Sullivan and Douglas, 1990), in which final BW and weight gain are highly correlated with feed intake, along with the correlation of percentage tibia ash and bone strength. Results from this study, however, indicated a stronger correlation between total tibia ash and bone strength than that suggested from previous studies (Potter et al., 1995; Ravindran et al., 1995). Growth and bone responses are correlated highest with available P intake as compared with dietary P levels, suggesting available P intake could be a used as an indicator of performance. This finding would only be valid if P was limiting in the diet at less than the available P requirement of 0.4% (National Research Council, 1984). As to be expected, total bone P and total bone Ca are highly correlated.

Extensive studies have found that natural phytate is a poor source of P for various species of poultry, but natural P in a finely ground whole wheat flour was almost completely available to chicks for growth but less available than inorganic phosphate for bone deposition (Sieburth et al., 1952). The apparent phytic acid digestibility determined in this study was higher in the barley diets (~39) compared to the corn diets (~25). Previous research demonstrated the amounts of phytic acid P hydrolyzed in barley is 32.2% and in corn is 30.8% (Leske and Coon, 1999). The results in this study show a similar trend except with younger birds. Therefore, barley may have more P available from phytate than corn, resulting in improved growth and bone response due to an inherent phytase factor.

Ca and Zn Availability

The results indicate no significant differences in apparent Ca and Zn digestibilities for the P source main effect (Tables 8 and 9). These results are somewhat contrary to previous findings, in which dietary phytate in sufficient quantity increased fecal excretion of Ca, Mg, and Zn (Morris, 1986). The relatively low phytate levels in this experiment may explain the contradictory results. Also, in some cases high-phytate treatments in Morris' review (1986) had higher mineral levels than low-phytate treatments. Higher levels of bone Ca content (Tables 6 and 7) were observed in birds fed low-phytate grain diets and P-supplemented wild-type diets compared to wild-type diets, probably due to the increased available P in the diets.

Higher levels of bone Zn content were observed using low-phytate grains compared to wild-type grain diets, probably due to the lower amounts of phytic acid in the diets and the amount of Ca absorbed in the low-phytate diets. High phytate intakes reduce Ca absorption and utilization of Ca in bone formation (Van Den Berg et al., 1972; Nwokolo and Bragg, 1977). Zinc homeostasis is partially maintained through reabsorption of endogenously secreted Zn from the intestine. Phytate may decrease endogenous Zn reabsorption as well as affect bioavailability of dietary Zn. The relationship between Ca, phytate, and Zn has been studied, and Ca appears to accentuate the decreasing effect of phytate on zinc availability (Wise, 1986). Calcium and Zn appear to have a synergistic effect in the precipitation of phytate by forming a Zn-Ca-phytate precipitate that binds the Zn more tightly than the Zn-phytate precipitate, making Zn less available for intestinal absorption (O'Dell et al., 1964).

Mg Availability

The results from this study agree with previous findings of a relationship between phytic acid and Mg (Atteh and Leeson, 1983). Diets prepared with low-phytate grains contained less phytic acid and relatively the same amount of Mg than wild-type grain diets and resulted in higher bone Mg content. Diets prepared with 60% grain contained more phytic acid and more Mg than 40% diets resulting in higher bone Mg content. However, P-supplemented wild-type diets had a similar amount of phytic acid but more available P than wild-type grain diets and resulted in higher bone Mg content. This result would suggest that the effects of phytic acid in diets on Mg availability might be offset by an increase in available P.

Summary

An improvement in growth and bone performance was observed due to increased available P when using the low-phytate grains over the wild-type grains. Correlations among percentage bone ash, total bone ash, and bone strength indicated a strong relation and appear to support the use of bone strength analysis as a simpler method than determination of percentage bone ash or total bone ash. The low-phytate grains investigated in this study will benefit the poultry industry by lowering the need for supplemental P, improving growth and bone performance of broilers, and decreasing P excretion into the environment.

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